Docket No.: 071949-5301
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Dahlen et al.

Title: Use of B-type Natriuretic Peptide as a Prognostic Indicator in Acute Coronary Syndromes

Appl. No.: 09/835,298

Filing Date: April 13, 2001

Examiner: Ann Y. Lam

Art Unit: 1641

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: **Commissioner for Patents, Alexandria, VA 22313**, on the date below.

Vanessa E. Ayna
(Printed Name)

Vanessa E Ayna
(Signature)

May 11, 2006
(Date of Deposit)

DECLARATION UNDER 37 C.F.R. 1.131

We, the undersigned named inventors of U.S. Patent Application 09/835,298, state and declare as follows:

1. We conceived and reduced to practice the invention claimed in the above-referenced U.S. Patent Application prior to April 10, 2001, and we provide evidence of same in the form of various pages from a draft of the above-referenced patent application. This draft was was prepared prior to April 10, 2001.

2. Our invention for determining a prognosis in a patient diagnosed with an acute coronary syndrome is described on page 3, last full paragraph, of the draft patent application. This paragraph is substantively repeated on page 3, second full paragraph, of the above-referenced U.S. Patent Application.

3. Our invention of prognostic methods, which includes prognosis of mortality, is described on page 4, last paragraph of the draft patent application. This paragraph is

substantively repeated on page 6, first full paragraph, of the above-referenced U.S. Patent Application.

4. Our invention of combining BNP measurements with cardiac troponin measurements in these prognostic methods is described on page 6 of the draft patent application. These paragraphs are substantively repeated on pages 8 and 9 of the above-referenced U.S. Patent Application.

5. Our invention of using a body fluid as a sample in these prognostic methods, including blood, serum, plasma, and urine, is described on page 7, first paragraph of the draft patent application. This paragraph is substantively repeated on page 9, third full paragraph, of the above-referenced U.S. Patent Application.

6. Our invention of using antibodies and various means for determining antibody binding to polypeptides such as BNP and cardiac troponin is described on page 12, first full paragraph, of the draft patent application. This paragraph is substantively repeated in the application as filed on page 15, second full paragraph, of the above-referenced U.S. Patent Application.

7. Our invention extends to prognosis of outcomes other than death, including subsequent myocardial infarction, onset of angina, and onset of congestive heart failure, is described on page 5, last paragraph, of the draft patent application. This paragraph is substantively repeated on page 8, first full paragraph, of the above-referenced U.S. Patent Application.

8. Actual reduction to practice of an embodiment of our claimed invention in the form of a working example that relates BNP and cardiac troponin measurements to prognosis of mortality in patients diagnosed with an acute coronary syndrome is described beginning on page 12 of the draft patent application. This example is substantively repeated in the above-referenced U.S. Patent Application beginning on page 16. In performing this example, blood specimens were obtained from subjects suffering from an acute coronary syndrome as described on page 13 of the draft patent application. Analytes, including cardiac troponin I and BNP, were measured

using standard immunoassay techniques. As described on page 18, first paragraph, of the draft patent application, cardiac troponin I and BNP were correlated to mortality rates across BNP quartiles. These results are presented as adjusted 10-month mortality in subjects stratified using 100 pg/mL cardiac troponin as a threshold in Figures 2 and 3. These figures are also repeated in the above-referenced U.S. Patent Application

9. From April 10, 2001 to April 13, 2001, we worked with outside counsel to revise the draft patent application.

11. We the undersigned further declare that all statements made herein of our own individual knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both under § 1001 of Capital Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

4/28/06
Date

Jeffrey R. Dahlen

5/4/06
Date

Gunars E. Valkirs

4/26/06
Date

Kenneth F. Buechler

[redacted]

DESCRIPTION

Use of B-Type Natriuretic Peptide as a Prognostic Indicator in Acute Coronary Syndromes

INTRODUCTION

The present invention relates in part to methods, compositions, and devices for the measurement of BNP, and the use of such measurement in the diagnosis, prognosis, and treatment of patients with acute coronary syndromes.

BACKGROUND OF THE INVENTION

The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

The term "acute coronary syndromes" ("ACS") has been applied to a group of coronary disorders that result from ischemic insult to the heart. Patients with ACS form a heterogeneous group, with differences in pathophysiology, clinical presentation, and risk for adverse events. Such patients present to the physician with conditions that span a continuum that includes unstable angina, non-ST-elevation non-Q wave myocardial infarction ("NSTMI"), ST-elevation non-Q wave MI, and transmural (Q-wave) MI. ACS is believed to result largely from thrombus deposition and growth within one or more coronary arteries, resulting in a partial or complete occlusion of the artery, and frequently involves rupture of the plaque, resulting in an ischemic injury. ACS may also be precipitated by a coronary vasospasm or increased myocardial demand. For review, *see, e.g.*, Davies, *Clin. Cardiol.* 20 (Supp. I): I2-I7 (1997).

The seriousness of ACS is underlined by the morbidity and mortality that follow the ischemic insult. For example, workers have estimated that within four to six weeks of presentation with ACS, the risk of death or a subsequent MI is 8-14%, and the rate of death, MI, or refractory ischemia is 15-25%. Theroux and Fuster, *Circulation* 97: 1195-1206 (1998) Given that the total number of deaths in the U.S. from acute MI is about 600,000, the search within the art for information that relates to the diagnosis, prognosis, and management of ACS has understandably been extensive. Several potential markers that may provide such information in

certain patient populations have been identified, including circulating cardiac troponin levels (*see, e.g.*, Antman *et al.*, *N. Eng. J. Med.* 335: 1342-9 (1996); see also U.S. Patent Nos. 6,147,688, 6,156,521, 5,947,124, and 5,795,725, each of which is hereby incorporated by reference in its entirety), ST-segment depression (*see, e.g.*, Savonitto *et al.*, *JAMA* 281: 707-13 (1999)), circulating creatine kinase levels (*see, e.g.*, Alexander *et al.*, *Circulation* (Suppl.) 1629 (1998)), and circulating c-reactive protein levels (*see, e.g.*, Morrow *et al.*, *J. Am. Coll. Cardiol.* 31: 1460-5 (1998)).

B-type natriuretic peptide ("BNP") is a 32-amino acid neurohormone that is synthesized in ventricular myocardium and released into the circulation in response to ventricular dilation and pressure overload. The functions of BNP, like atrial natriuretic peptide, include natriuresis, vasodilation, inhibition of the renin-angiotensin-aldosterone axis, and inhibition of sympathetic nerve activity. The plasma concentration of BNP is elevated among patients with congestive heart failure (CHF), and increases in proportion to the degree of left ventricular dysfunction and the severity of CHF symptoms. For review, *see, e.g.*, Wiese *et al.*, *Circulation* 102: 3074-9 (2000); Yasue *et al.*, *Circulation* 90: 195-203 (1994); Yoshimura *et al.*, *Circulation* 87: 464-9 (1993); and Stein and Levin, *Am. Heart J.* 135: 914-23 (1998).

Following the onset of acute MI, the plasma concentration of BNP has been shown to rise rapidly over the first 24 hours, and then to stabilize; patients with large infarcts may have a second peak in BNP concentration several days later. The concentration of BNP, when measured between 1 and 4 days following a transmural infarct, can provide prognostic information that is independent of the left ventricular ejection fraction (LVEF) and other important baseline variables. *See, e.g.*, Talwar *et al.*, *Eur. Heart J.* 21: 1514-21 (2000); Darbar *et al.*, *Am. J. Cardiol.* 78: 284-7 (1996); Richards *et al.*, *Heart* 81: 114-20 (1999); Omland *et al.*, *Circulation* 93: 1963-9 (1996); Arakawa *et al.*, *J. Am. Coll. Cardiol.* 27: 1656-61 (1996); and Richards *et al.*, *Circulation* 97: 1921-9 (1998).

To date, however, studies evaluating the prognostic implications of increased BNP concentration have been limited to patients with ST-elevation MI, and few data are available with regard to the prognostic implications of BNP following non ST-elevation acute coronary syndromes. Thus, there remains in the art the need to identify markers useful in evaluating

patient prognosis across the entire spectrum of acute coronary syndromes, so that patients at risk of near-term morbidity or and/or death or can be identified and treated.

SUMMARY OF THE INVENTION

The present invention relates to materials and procedures for evaluating the prognosis of patients suffering from acute coronary syndromes. In particular, the level of BNP in a patient sample, alone or in combination with one or more additional prognostic markers, can provide prognostic information useful for predicting near-term morbidity and/or mortality across the entire spectrum of acute coronary syndromes.

In various aspects, the invention relates to materials and procedures for identifying BNP levels that are associated with an increased predisposition to an adverse outcome in a patient; identifying one or more additional prognostic markers that increase the predictive value of a BNP level for such an adverse outcome; using the BNP level in a patient, alone or in combination with one or more additional prognostic markers, to determine a patient's prognosis; and using the BNP level in a patient, alone or in combination with one or more additional prognostic markers to determine a treatment regimen that improves a patient's prognosis.

Thus, the materials and procedures described herein can be used to identify those patients that are at acute risk for one or more serious complications, including the risk of death, resulting from acute coronary syndromes, and to guide the clinician in treatment of such patients.

In a first aspect, the invention relates to methods for determining the prognosis of a patient diagnosed with an acute coronary syndrome. These methods comprise identifying a BNP level that is associated with an increased predisposition of an adverse outcome resulting from an acute coronary syndrome. Once such a level is determined, the BNP level in a patient sample can be measured, and then compared to the diagnostic BNP level that is associated with the increased predisposition of the adverse outcome. By correlating the patient BNP level to the diagnostic BNP level, the prognosis of the patient can be determined.

The phrase "determining the prognosis" as used herein refers to methods by which the skilled artisan can predict the course or outcome of a condition in a patient. The term "prognosis" does not refer to the ability to predict the course or outcome of a condition with

100% accuracy, or even that a given course or outcome is more likely to occur than not. Instead, the skilled artisan will understand that the term "prognosis" refers to an increased probability that a certain course or outcome will occur; that is, that a course or outcome is more likely to occur in a patient exhibiting a given condition, when compared to those individuals not exhibiting the condition. In preferred embodiments, a prognosis is about a 5% chance of a given outcome, about a 7% chance, about a 10% chance, about a 12% chance, about a 15% chance, about a 20% chance, about a 25% chance, about a 30% chance, and about a 50% chance. Should we go higher in percentages?? The term "about" in this context refers to +/- 1%.

A prognosis is often determined by examining one or more "prognostic indicators." These are markers, the presence or amount of which in a patient (or a sample obtained from the patient) signal an increased probability that a given course or outcome will occur. For example, a preferred prognostic indicator in the present invention is BNP. As discussed herein, BNP is present in patients suffering from various acute coronary syndromes. When BNP reaches a sufficiently high level in samples obtained from such patients, the BNP level signals that the patient is at an increased probability for morbidity or death, in comparison to a similar patient exhibiting a lower BNP level. A BNP level that signals an increased probability for morbidity or death is referred to as being "associated with an increased predisposition to an adverse outcome" in a patient.

The term "correlating," as used herein in reference to the use of prognostic indicators to determine a prognosis, refers to comparing the presence or amount of the prognostic indicator in a patient to its presence or amount in persons known to suffer from, or known to be at risk of, a given condition; or in persons known to be free of a given condition. For example, a BNP level in a patient can be compared to a level known to be associated with an increased disposition for an MI or death. The patient's BNP level is said to have been correlated with a prognosis; that is, the skilled artisan can use the patient's BNP level to determine the likelihood that the patient is at risk for an MI or death, and respond accordingly. Alternatively, the patient's BNP level can be compared to a BNP level known to be associated with a good outcome (*e.g.*, no MI, no death, *etc.*), and determine if the patient's prognosis is predisposed to the good outcome.

In certain embodiments, a prognostic indicator is correlated to a patient prognosis by merely its presence or absence. For example, the presence or absence of ST-segment depression in an electrocardiogram can be correlated with a predisposition to certain conditions. See, *e.g.*, Savonitto *et al.*, *JAMA* 281: 707-13 (1999).

In other embodiments, a threshold level of a prognostic indicator can be established, and the level of the indicator in a patient sample can simply be compared to the threshold level. For example, a BNP level of 100 pg/mL in a patient sample can be established as a level at which a patient is at an increased disposition for morbidity or death. A preferred BNP threshold level of the invention is about 25 pg/mL, about 50 pg/mL, about 75 pg/mL, about 100 pg/mL, about 150 pg/mL, about 200 pg/mL, about 300 pg/mL, about 400 pg/mL, and about 500 pg/mL GREATER ?? The term "about" in this context refers to +/- 10%.

In yet other embodiments, a "nomogram" can be established, by which a level of a prognostic indicator can be directly related to an associated disposition towards a given outcome. The skilled artisan is acquainted with the use of such nomograms to relate two numeric values.

The phrase "acute coronary syndromes" as used herein refers to a group of coronary disorders that result from ischemic insult to the heart. ACS includes unstable angina, non-ST-elevation non-Q wave MI, ST-elevation non-Q wave MI, and transmural (Q-wave) MI. ACS can be divided into non-ST-elevation ACS and ST-elevation ACS, each of which may be associated with certain prognostic indicators and prognoses, as described herein. The phrase "non-ST-elevation acute coronary syndrome" refers to those ACS not associated with an elevated ST component in an electrocardiogram. Non-ST ACS include unstable angina and non-ST-elevation non-Q wave MI.

The phrase "adverse outcome" as used herein refers to morbidity or mortality suffered by a patient subsequent to the onset of ACS in the patient. For example, a patient may present to a clinician with ACS; an adverse outcome could be a subsequent MI, subsequent onset of angina, subsequent onset of congestive heart failure, or subsequent death. An adverse outcome is said to occur within the "near term" if it occurs within about 10 months of the onset of ACS.

In certain embodiments, one or more additional prognostic indicators can be combined with a patient BNP level to increase the predictive value of BNP as a prognostic indicator. The phrase "increases the predictive value" refers to the ability of two or more combined prognostic indicators to improve the ability to predict a given outcome, in comparison to a prediction obtained from any of the prognostic indicators alone. For example, a BNP level of X pg/mL may predict a 10% chance of a subsequent MI in the patient; and a cardiac troponin I level of Y ng/mL may predict a 5% chance of a subsequent MI. But the presence of both a BNP level of X pg/mL and a cardiac troponin I level of Y ng/mL in sample(s) obtained from the same patient may indicate a much higher chance of a subsequent MI in the patient. Preferred additional prognostic indicators of the invention are circulating cardiac-specific troponin levels, ST-segment depression, circulating creatine kinase levels, and circulating c-reactive protein levels.

The skilled artisan will understand that the plurality of prognostic indicators need not be determined in the same sample, or even at the same time. For example, one prognostic indicator may not appear in serum samples until some time has passed from the onset of ACS. Nevertheless, combining, for example, a cardiac troponin I level taken at 1 hour with a BNP level obtained at 48 hours, may provide the skilled artisan with an increased predictive value in comparison to either measurement alone.

Additionally, the increased predictive value need not be an increased probability of an adverse outcome. For example, a cardiac troponin I level taken at 1 hour may indicate a 5% chance of a subsequent MI. But when combined with a later BNP level that indicates a good prognosis in the patient, the result may be to reduce the predicted chance that the patient will suffer a subsequent MI.

The phrase "cardiac-specific troponin" refers to cardiac-specific isoforms of troponin I and T, and/or to complexes comprising at least one cardiac-specific troponin isoform. *See, e.g.*, U.S. Patent Nos. 6,147;688, 6,156,521, 5,947,124, and 5,795,725, each of which is hereby incorporated by reference in its entirety. Particularly preferred are methods that combine BNP and one or more cardiac-specific troponin isoforms as prognostic markers to determine the prognosis of a patient.

The term "patient sample" refers to a sample obtained from a living person for the purpose of diagnosis, prognosis, or evaluation. In certain embodiments, such a sample may be obtained for the purpose of determining the outcome of an ongoing condition or the effect of a treatment regimen on a condition. Preferred patient samples are blood samples, serum samples, plasma samples, cerebral spinal fluid and urine samples.

In another aspect, the invention relates to methods for determining a prognostic panel comprising a plurality of prognostic markers that can be used to determine the prognosis of a patient diagnosed with an acute coronary syndrome. These methods preferably comprise identifying a BNP level that is associated with an increased predisposition of an adverse outcome resulting from an acute coronary syndrome, and identifying one or more additional prognostic markers that increase the predictive value in comparison to that obtained from the use of BNP alone as a prognostic marker.

Once the plurality of markers has been determined, the levels of the various markers making up the panel can be measured in one or more patient sample(s), and then compared to the diagnostic levels determined for each marker, as described above.

In yet another aspect, the invention relates to methods for determining a treatment regimen for use in a patient diagnosed with an acute coronary syndrome. The methods preferably comprise determining a level of one or more prognostic markers as described herein, and using the prognostic markers to determine a prognosis for a patient. One or more treatment regimens that improve the patient's prognosis by reducing the increased disposition for an adverse outcome associated with the acute coronary syndrome can then be used to treat the patient.

In a further aspect, the invention relates to kits for determining the prognosis of a patient diagnosed with an acute coronary syndrome. These kits preferably comprise devices and reagents for measuring a BNP level in a patient sample, and instructions for performing the assay. Optionally, the kits may contain one or more means for converting a BNP level to a prognosis. Additionally, the kits may provide devices and reagents for determining one or more additional prognostic markers to be combined with a patient BNP level.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the association between BNP concentration and 10-month mortality. Patients were divided into quartiles based on the concentration of BNP at enrollment. Quartiles were recalibrated for each of the subgroups shown. STEMI = ST elevation myocardial infarction; NSTEACS = non ST elevation acute coronary syndrome; UA = unstable angina.

Figure 2 shows the association between baseline BNP concentration and 10-month mortality, stratified by the level of cardiac troponin I (cTnI) at the time of enrollment.

Figure 3 shows a stepwise logistic regression model showing the relationship between selected baseline clinical variables and 10-month mortality. Cardiac troponin I (cTnI) and BNP quartiles were forced into the final model. Odds ratios and 95% confidence intervals are shown. In addition to the variables shown in the figure, the final model included history of hyperlipidemia or peripheral vascular disease; prior therapy with diuretics, ACE inhibitors, nitrates, or heparin; heart rate; blood pressure; and creatinine clearance.

Figure 4 shows the numbers of patients in 3 adverse outcome groups (death, congestive heart failure (CHF), and myocardial infarction (MI)) at 30 days and 10 months, among patients with a BNP concentration above and below the prespecified threshold of 100 pg/mL.

Figure 5 shows the relationship between BNP concentration and 10-month mortality, using the prespecified threshold of 100 pg/mL to define BNP elevation. STEMI = ST elevation myocardial infarction; NSTEACS = non ST elevation acute coronary syndrome; UA – unstable angina.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Use of BNP as a prognostic marker in ACS

As demonstrated herein, the concentration of BNP, measured in the first few days after an acute coronary event, predicts the risk for morbidity and mortality across the entire spectrum of acute coronary syndromes. The prognostic utility of BNP persists after adjusting for clinical evidence of heart failure, as well as other important predictors of mortality, including clinical characteristics, ECG changes and cardiac troponin I.

Previous cohort studies have demonstrated that following acute MI, higher plasma concentrations of BNP and the N-terminal fragment of its prohormone (NT-pro BNP) are associated with larger infarct size (Arakawa *et al.*, *Cardiology* 85: 334-40 (1994); Horio *et al.*, *Am. Heart J.* 126: 293-9 (1993)), adverse ventricular remodeling (Nagaya *et al.*, *Am. Heart J.* 135: 21-8 (1998)), and lower ejection fraction and an increased risk for the development of congestive heart failure and death (Talwar *et al.*, *Eur. Heart J.* 21: 1514-21 (2000); Darbar *et al.*, *Am. J. Cardiol.* 78: 284-7 (1996); Richards *et al.*, *Heart* 81: 114-20 (1999); Omland *et al.*, *Circulation* 93: 1963-9 (1996); Arakawa *et al.*, *J. Am. Coll. Cardiol.* 27: 1656-61 (1996); Richards *et al.*, *Circulation* 97: 1921-9 (1998)). These prior studies have each included fewer than 150 patients, and focused on relatively homogenous groups of patients with ST elevation MI. Our study extends these findings in patients with non-ST elevation acute coronary syndromes including include unstable angina.

As demonstrated in the examples presented below, a single measurement of BNP, performed a median of 40 hours after the onset of ischemic symptoms, provides powerful risk-stratification across the entire spectrum of acute coronary syndromes. The prognostic implications of BNP levels are distinct from those of myocyte necrosis; that is, even among patients with unstable angina, the degree of BNP elevation is of prognostic significance.

Moreover, even after correcting for variables such as history of hypertension, heart failure, and prior diuretic or ACE inhibitor use, BNP remained predictive of long-term mortality. Thus, despite heterogeneity in pathophysiology and clinical presentation between patients with ST elevation MI, non-ST elevation ACS, and unstable angina, increasing BNP concentration was predictive of mortality in each of these subgroups, suggesting that activation of the cardiac neurohormonal system may be a unifying feature among patients at high risk for death across the entire spectrum of acute coronary syndromes.

The association between BNP and long-term mortality was independent of clinical evidence of congestive heart failure, as well as cardiac Troponin I, ECG changes, and other known predictors of mortality in ACS. In fact, BNP appeared to be a more powerful predictor of long-term mortality than any other variable measured. In addition, higher BNP levels were associated with an increased risk for the development of nonfatal endpoints, including new or

progressive heart failure and myocardial infarction. Finally, it appears that the previously defined BNP threshold of 100 pg/mL, indicative of neurohormonal activation in patients with congestive heart failure, also performs well among patients with ACS.

Also, unlike traditional cardiac biomarkers used to predict risk among patients with ACS, and particularly non-ST elevation ACS, BNP has a putative role in the counter-regulatory response to ischemic injury. As such, it may act as an index of the size or severity of the ischemic insult, as well as the degree of underlying impairment in left ventricular function. For example, in an animal model of transmural myocardial infarction, BNP gene expression was augmented 3-fold in the left ventricle within 4 hours after the onset of coronary artery ligation, and importantly, tissue concentrations of BNP were increased in non-infarcted as well as infarcted regions. Hama *et al.*, *Circulation* 92: 1558-64 (1995). Moreover, it has been demonstrated that BNP increases rapidly, and transiently, following exercise testing in patients with chronic stable angina, and that the degree of BNP elevation is closely correlated with the size of the ischemic territory as measured using nuclear SPECT imaging. Marumoto *et al.*, Clin. Sci. (Colch.) 88: 551-6 (1995).

Furthermore, BNP increases transiently following uncomplicated percutaneous transluminal coronary angioplasty even in the absence of changes in pulmonary capillary wedge pressure. Tateishi *et al. Clin. Cardiol.* 23: 776-80 (2000); Kyriakides *et al.*, *Clin. Cardiol.* 23: 285-8 (2000). Several small cross-sectional studies have shown that BNP and Nt-pro BNP concentrations are higher among patients with unstable angina than among patients with stable angina or among healthy controls. Talwar *et al.*, *Heart* 84: 421-4 (2000); Kikuta *et al.*, *Am. Heart J.* 132: 101-7 (1996). In one of these studies (Kikuta *et al.*), BNP elevation appeared to correlate with echocardiographic findings of regional wall motion abnormalities but not with hemodynamic data obtained at the time of simultaneous cardiac catheterization; furthermore, after medical stabilization, wall motion abnormalities improved and BNP levels fell significantly. Taken together, these prior studies suggest that myocardial ischemia may augment BNP synthesis and release, even in the absence of myocardial necrosis or pre-existing left ventricular dysfunction. Reversible ischemia may lead to a transient increase in left ventricular wall stress, which may be sufficient to cause BNP elevation.

Use of BNP for determining a treatment regimen

A useful prognostic indicator such as BNP can help clinicians select between alternative therapeutic regimens. For example, patients with elevation in cardiac troponin T or I following an acute coronary syndrome appear to derive specific benefit from an early aggressive strategy that includes potent antiplatelet and antithrombotic therapy, and early revascularization. Hamm *et al., N. Engl. J. Med.* 340: 1623-9 (1999); Morrow *et al., J. Am. Coll. Cardiol.* 36: 1812-7 (2000); Cannon *et al., Am. J. Cardiol.* 82: 731-6 (1998). Additionally, patients with elevation in C-reactive protein following myocardial infarction appear to derive particular benefit from HMG-CoA Reductase Inhibitor therapy. Ridker *et al., Circulation* 98: 839-44 (1998). Among patients with congestive heart failure, pilot studies suggest that ACE inhibitors may reduce BNP levels in a dose dependent manner. Van Veldhuisen *et al., J. Am. Coll. Cardiol.* 32: 1811-8 (1998).

Similarly, "tailoring" diuretic and vasodilator therapy based on NT-pro BNP levels may improve outcomes. Troughton *et al., Lancet* 355: 1126-30 (2000). Finally, in a single pilot study of 16 patients found that randomization to an ACE inhibitor rather than placebo following Q-wave MI was associated with reduced BNP levels over the subsequent 6-month period. Motwani *et al., Lancet* 341: 1109-13 (1993). Because BNP is a counter-regulatory hormone with beneficial cardiac and renal effects, it is likely that a change in BNP concentration reflects improved ventricular function and reduced ventricular wall stress. A recent article demonstrates the correlation of NT pro BNP and BNP assays (Fischer *et al., Clin. Chem.* 47: 591-594 (2001). It is a further objective of this invention that the concentration of BNP can be used to guide diuretic and vasodilator therapy to improve patient outcome. Additionally, this invention includes the measurement of NT-proBNP for use as a prognostic indicator for patients suffering from acute coronary syndromes.

Recent studies in patients hospitalized with congestive heart failure suggest that serial BNP measurements may provide incremental prognositic information as compared to a single measurement; that is, assays can demonstrate an improving prognosis when BNP falls after therapy than when it remains persistently elevated. Cheng *et al., J. Am. Coll. Cardiol.* 37: 386-91

(2001). Thus, serial measurements may increase the prognostic value in patients with non-ST elevation ACS as well.

Assay Measurement Srategies

Numerous methods and devices are well known to the skilled artisan for measuring the prognostic indicators of the instant invention. With regard to polypeptides, such as BNP, in patient samples, immunoassay devices and methods are often used. *See, e.g.*, U.S. Patents 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims. These methods and devices can utilize labeled molecules in various sandwich, competitive, or non-competitive assay formats, to generate a signal that is related to the presence or amount of an analyte of interest. Additionally, certain methods and devices, such as biosensors and optical immunoassays, may be employed to determine the presence or amount of analytes without the need for a labeled molecule. *See, e.g.*, U.S. Patents 5,631,171; and 5,955,377, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims.

Examples

The following examples serve to illustrate the present invention. These examples are in no way intended to limit the scope of the invention.

Example 1: Validation of BNP as a prognostic indicator in ACS

Study Population

The Oral Glycoprotein IIb/IIIa Inhibition with Orbofiban in Patients with Unstable Coronary Syndromes (OPUS-TIMI 16) Trial was a randomized multicenter trial comparing an oral glycoprotcin IIb/IIIa inhibitor, orbofiban, with placebo in 10,288 patients with acute coronary syndromes. Patients were included if they presented within 72 hours of the onset of ischemic discomfort and met one or more of the following criteria: dynamic ECG changes (ST deviation $\geq$ 0.5 mm, T-mm, T-wave inversion $\geq$ 3 mm in $\geq$ 3 leads, or left bundle branch block); positive cardiac markers; prior history of coronary artery disease; or age $\geq$ 65 with evidence of diabetes or vascular disease. *See, e.g.*, Cannon *et al.*, *Circulation* 102: 149-56 (2000).

The study population described herein consisted of a subpopulation of 2525 patients from the OPUS-TIMI 16 study, of whom 825 were enrolled following an index ST elevation MI, 565 following a non-ST elevation MI, and 1133 following a diagnosis of unstable angina. BNP concentration ranged from 0-1456 pg/mL, with a mean of 114 ± 3 pg/mL, a median of 81 pg/mL, and 25th and 75th percentiles of 44 and 138 pg/mL. Mean time from the onset of ischemic symptoms to randomization was 40 ± 20 hours (median 40 hours).

Blood Sampling

Blood specimens were collected by trained study personnel in citrate tubes and centrifuged for ≥ 12 minutes. The plasma component was transferred into a sterile cryovial and frozen at -20° C or colder.

Biochemical Analyses

Troponin I, CKMB, CRP and BNP were measured using standard immunoassay techniques. These techniques involved the use of antibodies to specifically bind the protein targets. CRP was measured using the N Latex CRP assay (Dade Behring) and fibrinogen was assayed using the Dade Behring Assay on the BN II analyzer. In the case of BNP measurements, an antibody, 106.3, was biotinylated using N-hydroxysuccinimide biotin (NHS biotin) at a ratio of about 5 NHS biotins per antibody. The 106.3-biotin conjugate was then added to wells of a standard avidin 384 well microtiter plate and antibody conjugate not bound to the plate was removed. This formed the 106.3 solid phase in the microtiter plate. Another antibody, BNP.5, was conjugated to alkaline phosphatase using standard techniques, using SMCC and SPDP. The immunoassays were performed on a TECAN Genesis RSP 200/8 Workstation. The plasma samples (10 µl) were pipeted into the microtiter plate wells, incubated for 60 min, the sample was removed and the wells were washed with a wash buffer, consisting of 20 mM borate (pH 7.42) containing 150 mM NaCl, 0.1% sodium azide, and 0.02% Tween-20. The BNP.5 alkaline phosphatase conjugate was then added to the wells and incubated for an additional 60 min, after which time, the antibody conjugate was remove and the wells were subsequently washed with a wash buffer. A substrate, (AttoPhos) was added to the wells, and the rate of formation of the fluorescent product was related to the concentration of the BNP in the patient samples.

Clinical Endpoints

All-cause mortality and nonfatal myocardial infarction were evaluated through 30 days, and the end of the follow up period (10 months). Myocardial infarction was defined using previously reported criteria based on CKMB elevation (Antman *et al.*, *Circulation* 100: 1593-

601 (1999)), and all cases of suspected myocardial infarction were adjudicated by a Clinical Events Committee. The endpoint of new or worsening CHF or cardiogenic shock was collected from case record forms and was not adjudicated.

Statistical Analyses

Subjects were divided into quartiles based on their concentration of BNP at the time of enrollment in the trial. Means and proportions for baseline variables were compared across quartiles using ANOVA for continuous variables and the $\chi 2$ trend test for categorical variables. The direct correlation between BNP and other continuous baseline variables was assessed using Pearson's test. Mean concentration of BNP was compared between patients who met a study endpoint and those who did not using the Student *t* test. Cox regression analysis was used to evaluate the association between increasing concentration of BNP and adverse cardiovascular outcomes through 30 days and 10 months. Stratified analyses were performed among patients with a cTnI level > 0.1 ng/ml and a cTnI ≤ 0.1 ng/ml, as well as those with and without a clinical diagnosis of congestive heart failure. Subgroup analyses were performed in groups defined by the following index diagnoses: ST elevation MI, non-ST elevation ACS, and unstable angina. Quartile ranges were recalculated for each of these subgroups. For the endpoint of all-cause mortality through the end of follow-up (10 months), a logistic regression model was constructed using forward stepwise selection. Clinical variables that were assessed in > 75% of the population were entered into the model, provided they had a univariate p value < 0.1; variables were removed from the model if they had a multivariate p value > 0.1. Baseline concentrations of cTnI and BNP were then forced into the completed model. Finally, analyses were performed using a single, prespecified BNP threshold of 100 pg/mL.

Association with Baseline Clinical Variables

In univariate analyses, higher baseline concentration of BNP was associated with older age, female sex, white race, and a prior history of hypertension, congestive heart failure, peripheral vascular disease, and cerebrovascular disease; BNP was inversely associated with history of hypercholesterolemia and current smoking (table 1). As expected, BNP levels were highest among patients with ST elevation MI, intermediate among patients with non-ST elevation MI, and lowest among those with unstable angina (table 1). Patients with higher BNP

concentrations were more likely to present in Killip Class II or greater, and were more likely to have ECG changes, elevations in cardiac biomarkers, and renal insufficiency (table 1).

Table 1
Baseline Clinical Characteristics According to Quartiles of BNP (pg/mL)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p trend	p Q4 vs. Q1
Range of BNP levels, pg/mL	0-43.6	43.7-81.2	81.3-137.8	137.9-1456.6		
n	631	632	632	630		
Time to randomization (hrs)	39 ± 21	40 ± 21	41 ± 20	41 ± 19	0.004	0.10
Age (years)	57 ± 10	59 ± 11	61 ± 12	66 ± 11	<0.0001	<0.0001
Male	474 (75%)	465 (74%)	472 (75%)	405 (64%)	0.0001	<0.0001
White	575 (91%)	592 (94%)	605 (96%)	603 (96%)	0.0002	0.001
Past Medical History						
Hypertension	246 (39%)	254 (40%)	263 (42%)	298 (47%)	0.003	0.003
Congestive Heart Failure	26 (4%)	28 (4%)	26 (4%)	56 (9%)	0.0006	0.0008
Coronary artery disease*	329 (52%)	312 (49%)	294 (47%)	327 (52%)	0.7	0.9
Peripheral vascular disease	33 (5%)	43 (7%)	48 (8%)	57 (9%)	0.008	0.009
Cerebrovascular disease	24 (4%)	32 (5%)	39 (6%)	60 (10%)	<0.0001	0.0001
Diabetes	138 (22%)	133 (21%)	132 (21%)	152 (24%)	0.4	0.3
Family history of CAD	268 (43%)	260 (41%)	253 (41%)	232 (37%)	0.045	0.04
Hypercholesterolemia	199 (32%)	191 (30%)	173 (28%)	149 (24%)	0.0009	0.002
Smoking status:						
Current smoker	233 (37%)	263 (42%)	236 (38%)	189 (30%)		
Never smoker	193 (31%)	161 (26%)	185 (29%)	254 (40%)		
Past smoker	204 (32%)	205 (33%)	209 (33%)	186 (30%)		
Index Diagnosis:					<0.0001	<0.0001
ST elevation MI	141 (22%)	189 (30%)	231 (37%)	264 (42%)		
Non ST elevation MI	87 (64%)	137 (22%)	159 (25%)	182 (29%)		
Unstable angina	402 (64%)	306 (48%)	241 (38%)	184 (29%)		
Physical findings						
BMI kg/m^2	29 ± 5	28 ± 5	28 ± 14	28 ± 12	0.1	0.08
Systolic BP (mm Hg)	130 ± 20	129 ± 19	128 ± 22	129 ± 21	0.3	0.4
Killip Class II-IV	31 (5%)	36 (6%)	56 (9%)	109 (18%)	<0.0001	<0.0001
Diagnostic Testing						
Creatinine clearance ≤ 90	146 (24%)	185 (31%)	229 (38%)	350 (58%)	<0.0001	<0.0001
CK-MB >ULN	212 (58%)	308 (72%)	349 (79%)	388 (86%)	<0.0001	<0.0001
ST depression > 0.5mm	270 (43%)	297 (47%)	311 (49%)	356 (57%)	<0.0001	<0.0001
T wave inversion > 3mm	137 (22%)	146 (23%)	171 (27%)	167 (27%)	0.02	0.047

* CAD = Prior coronary artery disease; previous MI, documented unstable angina, angina pectoris, angiographically confirmed CAD, prior PTCA or CABG not for index event. MI=myocardial infarction;; BMI=Body Mass Index; ULN=upper limit of normal

Although statistically significant, the associations between the baseline concentration of BNP and C-reactive protein (R=0.2; p<0.0001), Fibrinogen (R=0.18; p<0.0001), peak recorded CK-MB (R=0.09; p=0.0005) and LVEF (R=0.23; p<0.0001) were only modest. Results from

coronary arteriography, echocardiography, and exercise stress testing were available in a subset of the patient population. Higher BNP concentration was associated with more severe coronary disease, lower ejection fraction, and a positive exercise stress test ($p<0.01$ for each; table 2).

Table 2. Association between cardiac test results and BNP concentration

Test	Result	n	BNP (Mean ± SD)	p value
Coronary Angiography:	None	27	68 ± 48	<0.0001
No. vessels with ≥	1	220	83 ± 65	
50% stenosis	2	106	98 ± 98	
	≥ 3	79	143 ± 145	
LV Ejection Fraction	≤ 50%	156	136 ± 161	0.003
	> 50%	189	96 ± 78	
Stress test	Positive	296	118 ± 118	0.003
	Indeterminate	118	118 ± 128	
	Negative	374	91 ± 95	

LV=left ventricular; SD=standard deviation

Clinical Outcomes

Mean concentration of BNP was significantly higher among patients who died by 30 days ($p<0.0001$) or by 10 months ($p<0.0001$) vs those who were alive at either time point (table 3). These differences remained significant in subgroups of patients with ST elevation MI, non-ST elevation ACS, and unstable angina ($p<0.01$ for each subgroup at both 30 days and 10 months; table 4). Mean BNP levels were significantly higher among patients with a myocardial infarction by 30 days ($p=0.01$) or 10 months ($p=0.02$), as compared with patients free of MI at these time points (table 3). Finally, BNP concentration was higher among patients who developed new or worsening CHF by 30 days (p—x) or 10 months (p—y) than among those who

did not develop CHF (table 3)[redacted]

Unadjusted mortality increased in a stepwise direction across increasing quartiles of baseline BNP concentration ($p<0.0001$: figure 1). These differences remained significant in subgroups of patients with ST elevation MI, non-ST elevation ACS, and unstable angina ($p\leq0.02$ for each; figure 1). In addition, the relationship between BNP and 10-month outcomes remained graded and significant both among patients with and those without history or exam evidence of CHF at enrollment (table 4).

Table 4. Association between baseline BNP concentration (pg/ml) and 10-month outcomes in subgroups based on index diagnosis.

Outcome	n	Median [25,75]	Mean ±SD	p value
ST elevation MI	825	96 [56,161]	131 ± 4	
Dead by 30 days	13	153 [77,265]	236 ± 220	0.002
Alive at 30 days	812	95 [56,161]	[redacted]	
Dead by 10 months	23	150 [90,227]	199 ±176	0.008
Alive at 10 months	802	95 [55,161]	129 ± 123	
Non-ST elevation ACS	1698	72 [39,124]	106 ± 3	
Dead by 30 days	26	149 [84,307]	220 ± 200	<0.0001
Alive at 30 days	1672	71 [39,123]	105 ± 124	
Dead by 10 months	62	142 [88,320]	239 ± 245	<0.0001
Alive at 10 months	1636	70 [38,121]	101 ± 117	
Unstable Angina	1133	60 [33,105]	92 ± 3	
Dead by 30 days	14	94 [69,237]	182 ± 195	0.002
Alive at 30 days	1119	60 [33,105]	90 ± 109	
Dead by 10 months	34	96 [70,265]	233 ± 292	<0.0001
Alive at 10 months	1099	58 [33,104]	87 ± 97	

MI=myocardial infarction; SD=Standard deviation

When stratification was performed based on the concentration of cTnI at the time of enrollment, increasing BNP concentration remained associated with higher 10-month mortality, both among those with a cTnI ≤ 0.1 ng/mL (n=882; p=0.01) and those with a cTnI > 0.1 ng/mL (n=1630; p<0.0001) (figure 2). After adjustment for other independent predictors of long-term mortality, including ST deviation and cTnI, increasing concentration of BNP remained associated with a higher rate of death by 10 months (figure 3). The adjusted odds ratios for 10-month mortality were 3.9 (1.1-13.6), 4.3 (1.3-15.0), and 6.7 (2.0-22.6) for patients with BNP concentrations in the second, third, and fourth quartile, respectively (figure 3).

Evaluation of 100 pg/mL BNP Threshold

Analyses were performed using a prospectively defined BNP threshold of 100 pg/mL. Patients with a plasma concentration of BNP > 100 pg/mL were significantly more likely to suffer death, myocardial infarction, or new/progressive CHF than those with a BNP level ≤ 100 pg/mL (p<0.005 for each at 30 days and 10 months; figure 4). In subgroups of patients with ST elevation MI, non-ST elevation ACS, and unstable angina, a BNP level > 100 pg/mL was associated with a significant increase in the risk for 10-month mortality (figure 5).

While the invention has been described and exemplified in sufficient detail for those skilled in this art to make and use it, various alternatives, modifications, and improvements should be apparent without departing from the spirit and scope of the invention.

One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The processes and methods are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention and are defined by the scope of the claims.

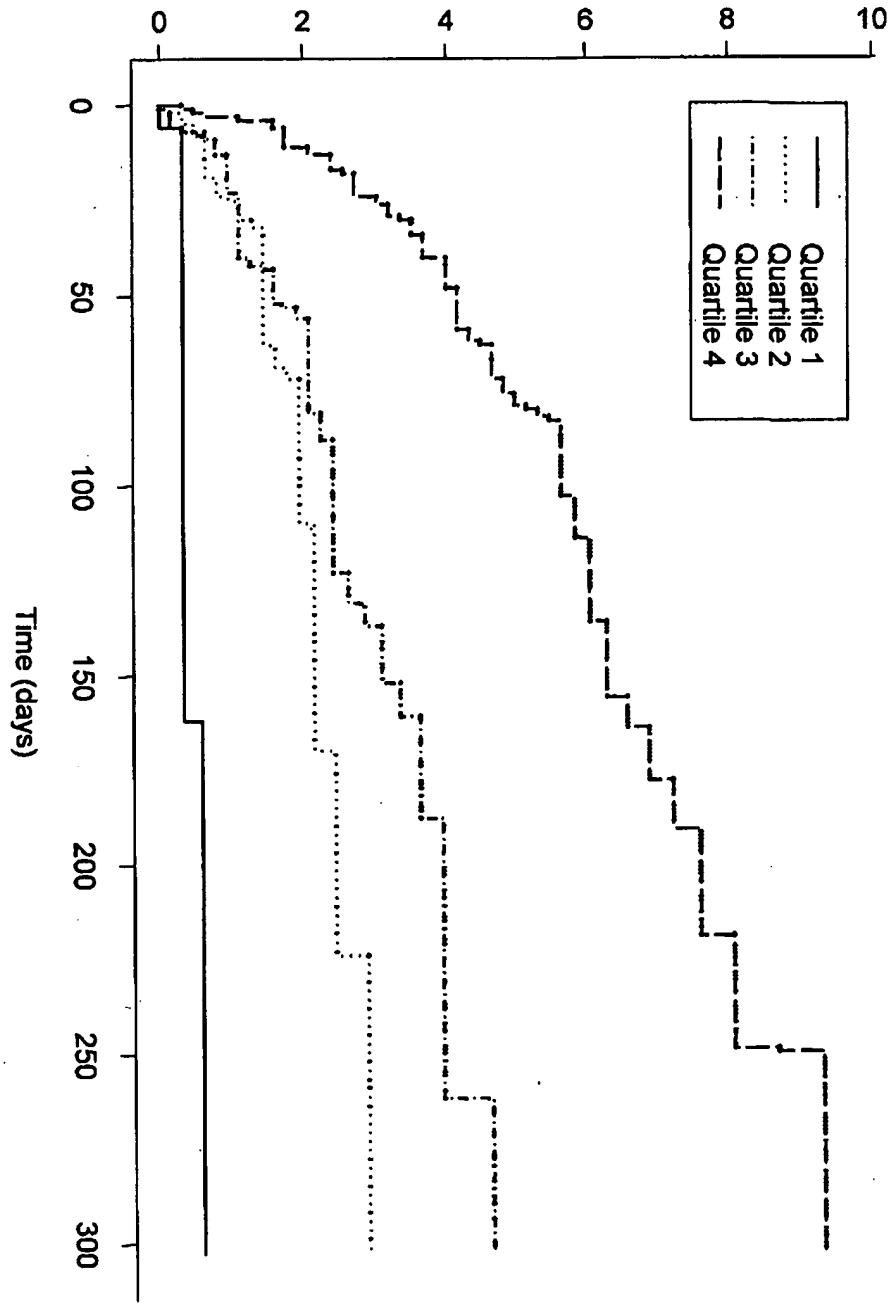
It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

Other embodiments are set forth within the following claims.

Fig 1



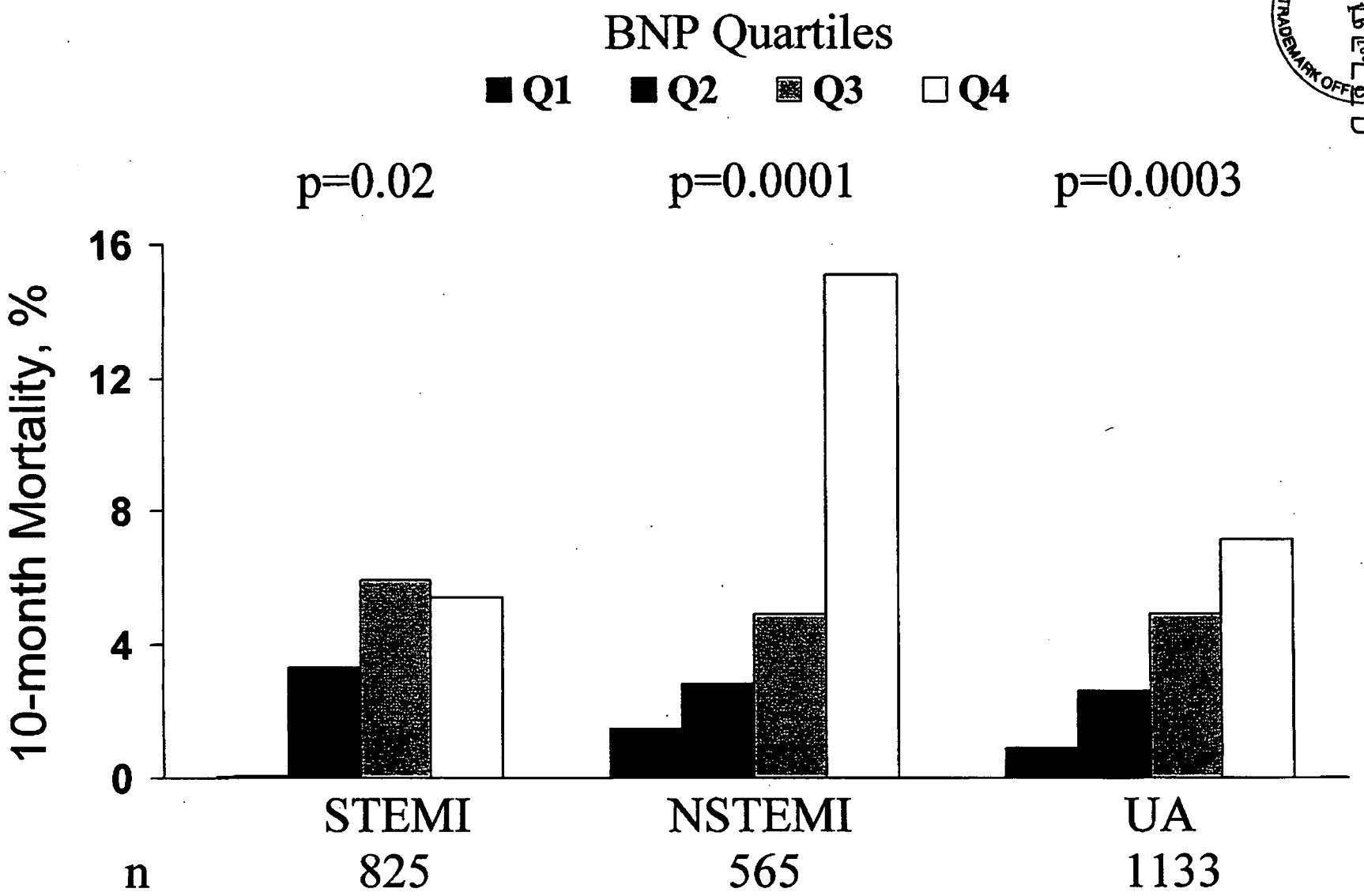


Fig 2

Adjusted 10-month Mortality

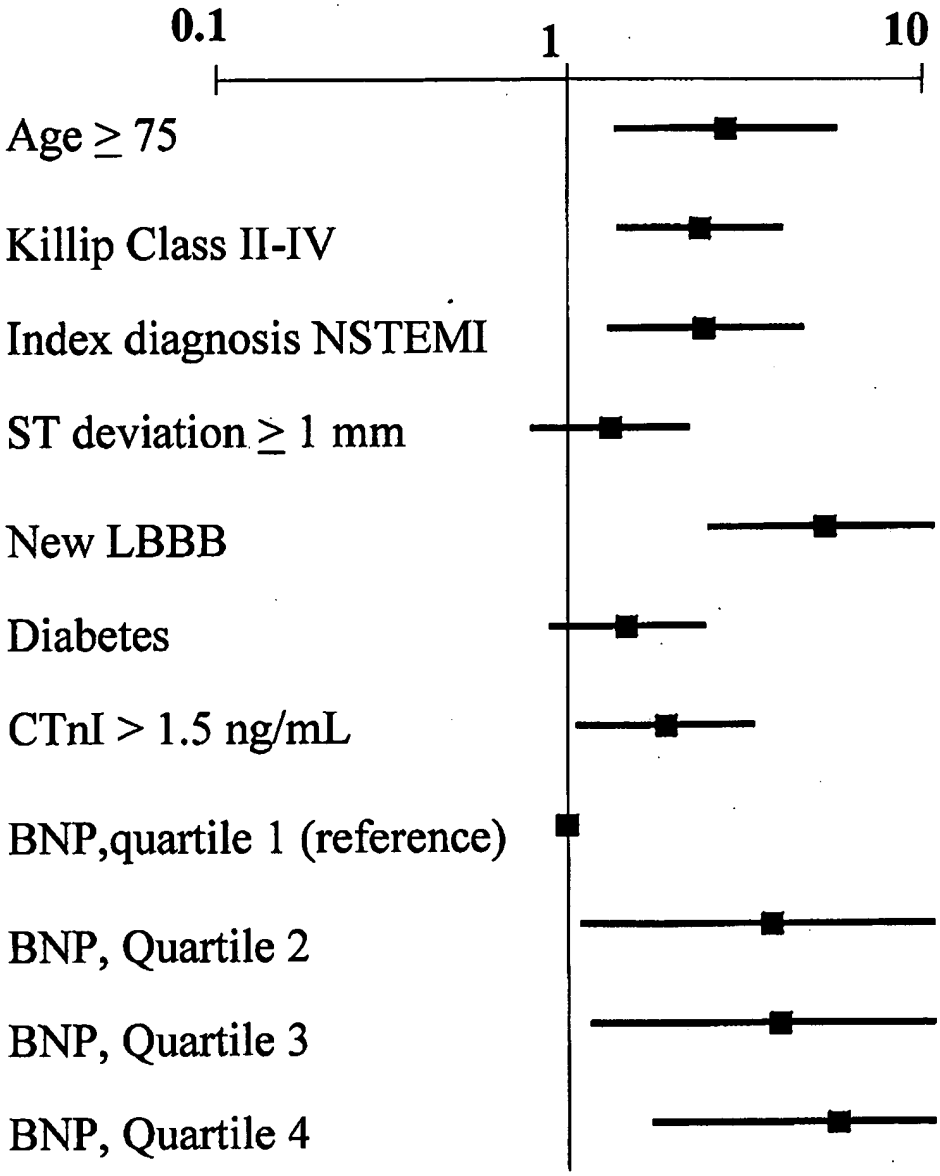


Fig 3

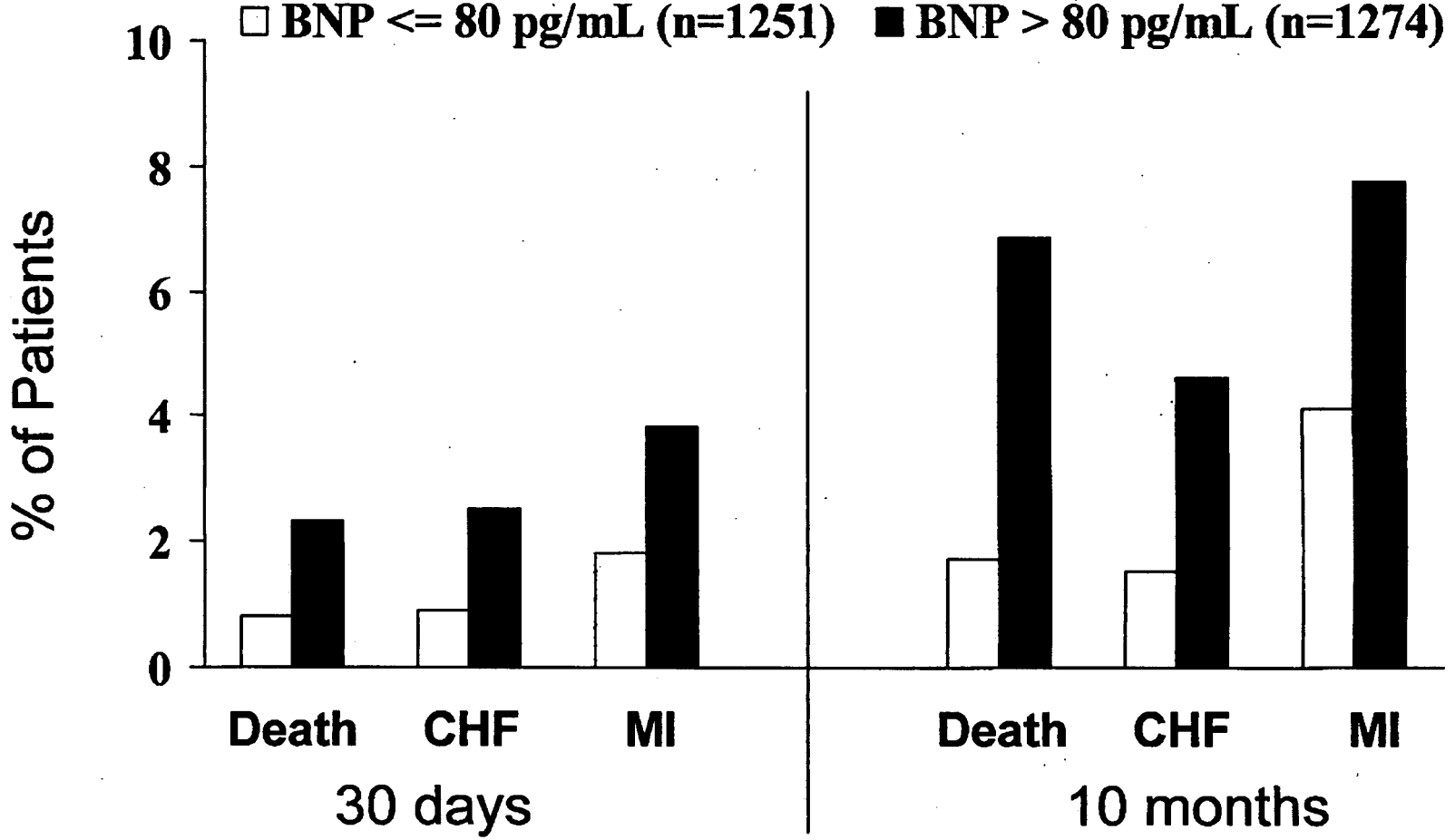


Fig 4

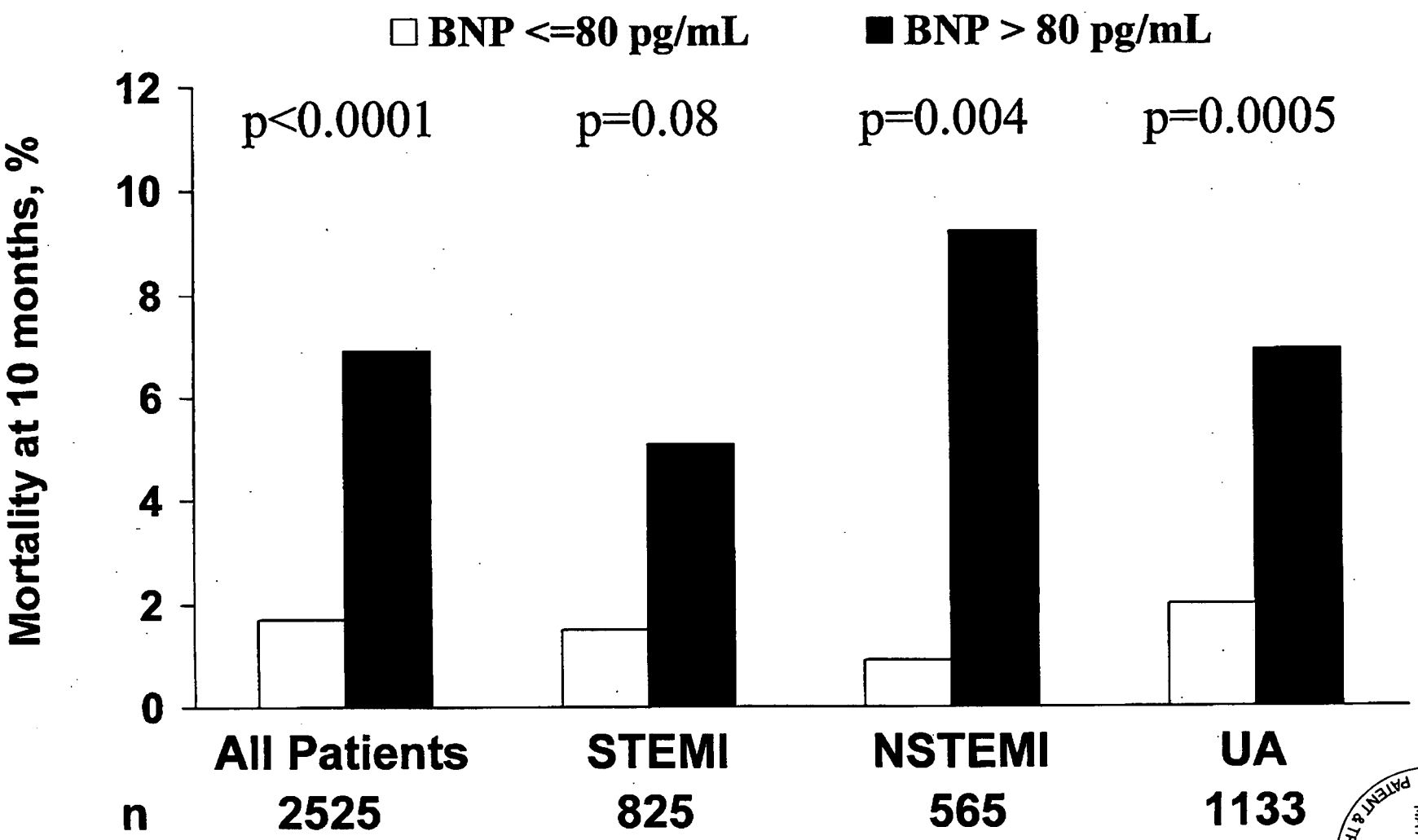

□ BNP <=80 pg/mL
■ BNP > 80 pg/mL
Mortality at 10 months, %
12
10
8
6
4
2
0
p<0.0001
p=0.08
p=0.004
p=0.0005
All Patients
STEMI
NSTEMI
UA
n
2525
825
565
1133

Fig 5